Anisotropy, age and gender dependence of cortical bone revealed by quantitative scanning acoustic microscopy

Kay Raum, Lüdger Smitmans and Jörg Brandt
Q-BAM, Medical Faculty, Martin Luther University of Halle, Germany; Email: kay.raum@medizin.uni-halle.de

Introduction

Bone is a heterogeneous and anisotropic material, which is composed of several levels of hierarchical organization. Low frequency ultrasound has been extensively used for the characterization of the macroscopic elastic properties. By increasing the frequency up to the gigahertz range it is possible to investigate the mesoscopic and the microscopic levels of structural organization. The acoustic impedance is as a tissue parameter, which is closely related to its elasto-mechanical properties. A major requirement for a quantitative analysis is a well prepared, flat surface. However, mechanical preparation techniques will remove softer materials more easily than stiffer ones, which will always lead to a remaining surface roughness in heterogeneous materials with varying mechanical properties. These contrast influences, caused by defocus, edges and surface inclinations, respectively are either compensated or excluded from the measurement using the *Multi Layer Analysis* technique for data acquisition and evaluation [1-5].

Experimental Setup

A scanning acoustic microscope (KSI SAM 2000, Kraemer Scientific Instruments, Herborn, Germany) with a broadband lens (0.8 – 1.3 GHz, aperture angle of 100°) was operated at 900 MHz in burst mode. The measurements were made at room temperature using distilled, degassed water as a coupling fluid. These conditions ensured a lateral resolution of approximately 1.5 µm, which is comparable to the minimum thickness of alternating osteon lamellae.

Proximal cortical bone sections were obtained from 26 human cadaver femora (17 male,9 female; age between 20 and 79 years, without bone disease in history) approximately 10 cm beneath of the femoral head. After dehydration the specimen were embedded in PMMA. The specimens were cut in various directions in order to obtain flat surfaces with orientations of 0, 10, 15, 30, 45, 60 and 90 degrees relative to the long axis of bone (Fig. 1a). The surfaces were then grinded and polished. From each sample peripheral, medial and central areas with scan sizes of (0.5 mm)² were measured (Fig. 1b). Impedance histograms of the whole scanned area and three characteristic structures, namely one completely developed osteon, one region of interstitial lamellae with apparent high reflectance and one region with relatively low reflectance, respectively, were chosen for statistical analysis (see Fig. 1).

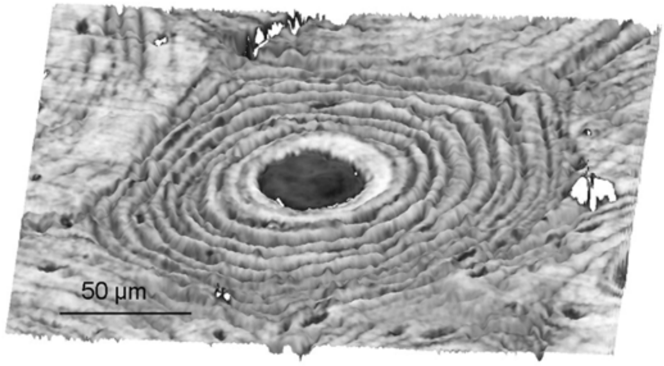
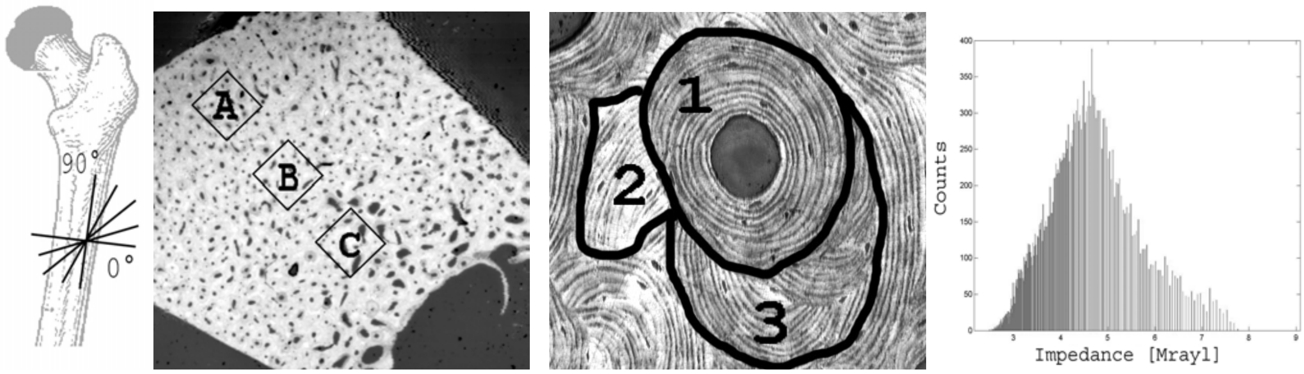


Figure 2: 3D reconstruction of a cortical bone sample. The Haversian channel is surrounded by alternating mineralized osteon lamellae. Adjacent to the osteon fractions of secondary osteons can be seen. The small dark spots are the hosts of the osteocytes.

Results

Fig. 2 shows a typical example of a cross section of compact bone. In the center there is an osteon with the Haversian channel (which is completely filled with the embedding material) and alternating mineralized collagen lamellae. Adjacent to the osteon parts of so-called secondary osteons are visible. Large variations of the reflectivity can be particularly seen in the osteon. It should be noted that higher values in the maximum image are well correlated with an surface elevation.

The mean impedance of all investigated samples was 3.38 Mrayl (std: 0.37 Mrayl). A significant anisotropy was found, whereat the maximum occurred at 10° (3.54 Mrayl, std: 0.35 Mrayl) and the minimum at 90° (3.22 Mrayl, std: 0.30 Mrayl), respectively (Fig. 3a). The angular differences were significant for most of the angle combinations (e.g. 10° vs. 90°, $p < 0.001$, paired t-test), except for directly adjacent angles. The same behavior was found for the gender specific subgroups. Gender specific differences of the mean impedance were found by one-way ANOVA testing at 10° ($p = 0.001$), 15° ($p = 0.006$) and 60° ($p = 0.006$), respectively. Fig. 2b shows the age and gender dependence after dividing the data set into the following subgroups: premenopausal (below 40 years), perimenopausal (between 40 and 60 years), postmenopausal (between 60 and 70 years) and senium (above 70 years). In addition to the general increase of impedance with age



a) b) c) d)

Figure 1: Selection of the samples (a), areas (b) and structural regions (c) for the investigation. Areas filled with the embedding material, e.g. in the center of region 1, were excluded from the histogram evaluation.

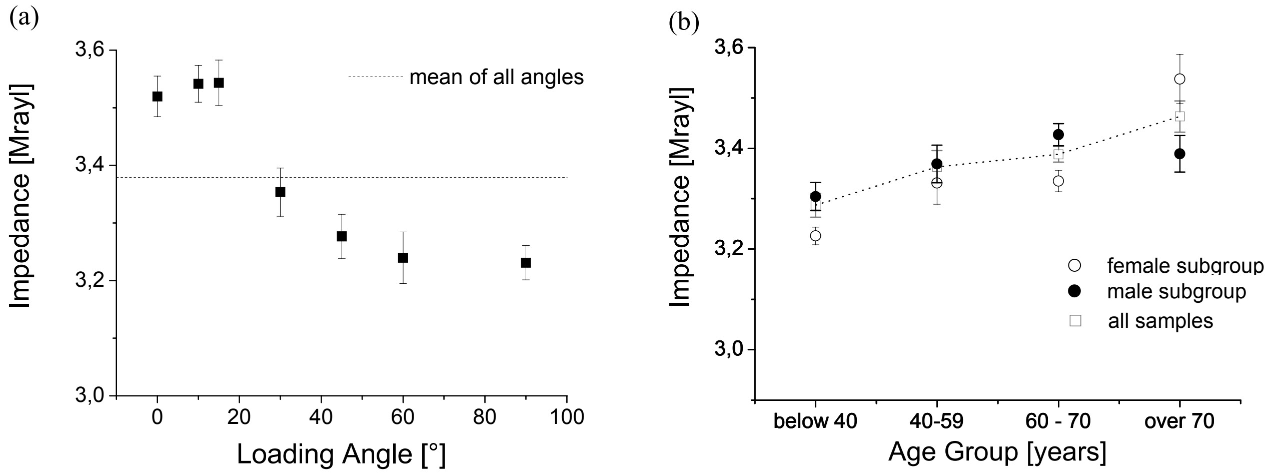


Figure 3: Angular (a) and age (b) dependence of the acoustic impedance (mean and standard error).

(correlation coefficient R= 0.981) the female samples exhibited a slightly lower impedance than the male samples (1.1 - 2.7 %), except for the senium group.

A comparison of the three selected histological structures (osteons, interstitial lamellae with high and low reflectance) revealed a similar anisotropic behavior for all structures. The only difference appeared in the absolute values. While the angular dependence of osteons agrees well with that of the overall measured regions (within 1%), the impedance is approximately 21 % higher for interstitial lamellae with high reflectance and 12 % lower for those with low reflectance, respectively.

Conclusions

The observed high resolution impedance values at 900 MHz were generally lower than low frequency bulk values [6]. A possible explanation might be a structural dependent sound dispersion, which has been reported previously in the frequency range from 5 to 100 MHz by Lees et al. [7] in cow bone, where a maximum was found at 70 MHz.

The angular dependence of the acoustic impedance shows trends similar to an orthotropic model for the Young's modulus proposed by Wagner and Weiner [8]. Experimentally the angular dependence of stiffness was derived from sound velocity measurements at 50 MHz by Pidaparti et al. [9] in canine femoral bone and in mongrel dog femora by Turner et al. [10]. The latter used low frequency ultrasound of 2.25 and 50 MHz. In native femora samples they found a maximum anisotropy at off-axis angles from 0° to 30° and minimum anisotropy at 60° to 70°. This result is analogous to the findings of the present investigation, although the difference in loading forces in-vivo for animal and human bone samples limit the expressiveness of a direct comparison. Furthermore the resolution in these studies was only about 60 µm. Moreover a good correlation was observed via a direct comparison of the acoustic impedance values and the Young's modulus revealed by nanoindentation [11]. This implies that high resolution acoustic impedance mapping is a powerful tool for the elastic characterization of bone microstructure.

Further investigations will focus on the gender specific characteristics and will include pathological samples. It is hoped, that deviations from the acousto-mechanical behavior presented in this study can help to improve our understanding of structure-mechanical relations.

References

[1] Raum,K., Brandt,J., Klemenz,A., and Cobet ,U., "Quantitative Ultraschallrastermikroskopie zur Bestimmung der akustischen Impedanz von kortikalem Knochengewebe," *Z. Med. Phys.*, vol. 9, pp. 246-253, 1999.

[2] Raum,K., Cobet ,U., Smitmans,L., and Brandt,J., "Quantitative scanning acoustic microscopy of cortical bone using a multi layer analysis method," in *Proc. IEEE Ultrason. Symp.*, 1999, pp. 593-596.

[3] Smitmans,L., Raum,K., Brandt,J., and Klemenz,A., "Variations in the microstructural acousto-mechanical properties of cortical bone revealed by a quantitative acoustic microscopy study," in *Proc. IEEE Ultrason. Symp.*, 2001, pp. 1379-1382.

[4] Raum,K., Jenderka,K.V., Klemenz,A., and Brandt,J., "Multi layer analysis - quantitative scanning acoustic microscopy for tissue characterization at a microscopic scale," *IEEE Trans. Ultrason. , Ferroelect. , Freq. Contr.*, in press, 2002.

[5] Raum,K., Jenderka,K.V., Klemenz,A., and Brandt,J., "Topographically corrected acoustic impedance estimation in cortical bone at a microscopic scale," in *Proc. IEEE Ultrason. Symp.*, in press, 2002.

[6] Raum,K., Reißhauer,J., and Brandt,J., "Frequency dependence of the anisotropic impedance estimation in cortical bone using time-resolved scanning acoustic microscopy," in *Proc. IEEE Ultrason. Symp.*, in press, 2002.

[7] Lees,S. and Klopholz, "Sonic velocity and attenuation in wet compact cow femur for the frequency range 5 to 100 MHz," *Ultrasound Med. Biol.*, vol. 18, pp. 303-308, 1992.

[8] Wagner,H.D. and Weiner,S, "On the relationship between the microstructure of bone and its mechanical stiffness," J. Biomech., vol. 25, pp.1311-1320, 1992.

[9] Pidaparti,R.M.V., Chandran,A., Takano,Y., and Turner,C.H., "Bone mineral lies mainly outside collagen fibrils: predictions of a composite model for osteonal bone," J. Biomech., vol. 29, 909-916, 1996.

[10] Turner,C.H., Chandran,A., and Pidaparti,R.M.V., "The anisotropy of osteonal bone and its ultrastructural implications," Bone, vol. 17, pp. 85-89, 1995.

[11] Brandt,J., Klemenz,A., Raum,K., and Seidler,S., "Scanning acoustic microscopy for micromeasurement of elastomechanical bone properties - comparison with nanoindentation results," *Proc. Int. Acoust. Imag. Symp.*, vol. 26, pp. 10, 2001.